Final Report

Original 2 of 2

Determination of the ~~aerobe ready~~ biodegradability of [redacted]

in the CO_2 Evolution Test
following OECD 301B resp. EU C.4.C

Study No.: [redacted]

Sponsor:
[redacted]

Test Facility:
[redacted]

Monitor:
[redacted]

Study Director:
[redacted]

This page was intentionally left blank for statement of sponsor or submitter.

1 GLP-Compliance Statement

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) §19a and §19b and annexes 1 and 2 in the version of 02 July 2008 published in Bundesgesetzblatt No. 28/2008, pp. 1146 - 1184

Responsibility for the accuracy of the information concerning the test item as well as for its authenticity rests with the sponsor.

I herewith accept responsibility for the data presented within this report.

There were no circumstances that may have affected the quality or integrity of the study.

[redacted]

Study Director

[redacted]

Date

Information on Study Organisation:

Deputy Study Director

Study Plan dated

Experimental Starting Date

Experimental Completion Date

Draft Report dated

[redacted]

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Study Plan and Final Report were checked at the dates given below, the Study Director and the management were informed with the corresponding report.

Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.

The study was conducted and the reports were written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Study Plan were acknowledged and assessed by the Study Director and included in the Final Report.

The reported results reflect the raw data of the study.

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Study plan	[redacted]		
Performance of study			
Draft report			
Final report			

[redacted]

Quality Assurance Manager

[redacted]

Date

Table of Contents

3 SUMMARY

Title of Study: Determination of the aerobe ready biodegradability of [redacted] in the CO_2 Evolution Test following OECD 301B resp. EU C.4.C

Findings and Results:

The test item [redacted] was tested using a concentration of nominally 20 mg organic carbon/L (corresponding to 137.0 mg [redacted] /L) in test medium following OECD 301B and EU-Method C.4-C..

Aniline was chosen as positive control.
Activated sludge was used as inoculum (concentration in the test 25 mg dry matter/L). The test was left running for 28 days.
All validity criteria were met. Degradation of the positive control was 71 % after nine days.

The following data were determined for the test item [redacted]

10-day-window:	not detected
degradation at the end of 10-day-window	none
degradation at the end of the test	no degradation
pass level:	60% at the end of 10-day-window

Therefore, [redacted] is **not readily biodegradable** following OECD 301B/EU C.4-C.

4 PURPOSE OF THE STUDY

This study was performed in order to evaluate aerobic elimination and degradation potential of [redacted] in a test for ready biodegradability, using a test item concentration of nominally 20 mg organic carbon/l (corresponding to 137.0 mg [redacted].
Sponsor's intent: REACH.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guidelines for the Testing of Chemicals Part 301 B, adopted 17. Jul. 1992 "Ready Biodegradability - CO_2-Evolution (Modified Sturm Test)"
- Commission Regulation (EC) No. 440/2008, Method C.4-C, adopted 31. May 2008 "Determination of Ready Biodegradability - Carbon Dioxide (CO_2) Evolution (Modified Sturm Test)"

Corresponding SOP of [redacted]

- [redacted]

6 MATERIALS AND METHODS

6.1 Test Item

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name
Batch no.
Appearance
Composition
CAS No.
EINECS-No.
Molecular formula
Molecular weight
Purity
Homogeneity
Volatility
Stability
Solubility
Production date
Expiry date
Storage
Hazard information
R-phrases
S-phrases

[redacted]

6.1.2 Storage
The test item was stored in a tightly closed glass vessel at room temperature under dry conditions in the dark.

6.1.3 Pre-Treatment
Taking into account the [redacted] the test item was added to the flasks as solid according to the nominal amount of organic carbon from the molecular formula [redacted] organic carbon).

6.2 Positive Control

Aniline (Phenylamine, $C_6H_5NH_2$, CAS-No. 62-53-3) was used as readily bio-degradable positive control. A stock solution containing 2.1 g/L (nominal) in deionised water was prepared and its organic carbon content was measured with 1668 ppm, resulting in an organic carbon content of the positive control of 79.4 %.

6.3 Test System

6.3.1 Specification
Activated sludge from a biologic sewage treatment plant was used. The chosen plant is treating mostly domestic sewage.

6.3.2 Source and Pre-Treatment

6.3.2.1 Source
The sludge was taken from the activation basin of the ESN (Stadtentsorgung Neustadt) sewage treatment plant, Im Altenschemel, NW-Lachen-Speyerdorf.

Date of collection: 15. Oct. 2010, batch no: 20101015.

6.3.2.2 Pre-Treatment
The sludge was filtrated, washed with tap water twice, then washed with and re-suspended in test medium. It was then aerated for ≥ 12 hours. The dry matter was determined with 4540 mg suspended solids/litre.

6.4 Chemicals

All chemicals used in the test were "analytical grade" or otherwise proved suitable.

6.4.1 Stock solutions

6.4.1.1 Solution a

Potassium dihydrogenephosphate (KH_2PO_4)	8.5 g
Di-potassium hydrogenephosphate (K_2HPO_4)	21.75 g
Di-sodiumhydrogenephosphate dihydrate ($Na_2HPO_4*2H_2O$)	33.4 g
Ammonia chloride (NH_4Cl)	0.5 g
H_2O demin. ad	1000 mL

The pH was adjusted to 7.4 ± 0.1.

6.4.1.2 Solution b

Calcium chloride dihydrate ($CaCl_2*2H_2O$)	36.4 g
H_2O demin. ad	1000 mL

6.4.1.3 Solution c

Magnesium sulfate heptahydrate ($MgSO_4*7H_2O$)	22.5 g
H_2O demin. ad	1000 mL

6.4.1.4 Solution d

Iron(III) chloride hexahydrate ($FeCl_3*6H_2O$)	0.25 g
Di-sodium-ethylendiamintetraacetate dihydrate ($Na_2EDTA*2H_2O$)	0.4 g
H_2O demin. ad	1000 mL

6.4.2 Test Medium

The medium was freshly prepared.

Composition:

Solution a	10 mL
Solution b	1 mL
Solution c	1 mL
Solution d	1 mL
H_2O demin. ad	1000 mL

6.5 Test Vessels

All glassware was cleaned with the laboratory cleaning agent Mucasol® and then rinsed with tap water (thrice), diluted HCL (once), tap water (thrice) and deionised water (thrice).

2000 mL-SCHOTT-flasks were used as test vessels, 100 mL scrubber flasks as absorbent vessels.

6.6 Instruments and Devices

The following instruments and devices were used in the performance of the study:

- Data logger for temperature
- Analytical scales Mettler Toledo AB 184 SA
- Analytical scales Mettler Toledo XS DU 205 No. 1
- Precision scales Sartorius 1403
- Adjustable pipettes with one-way tips Rainin®; [redacted]
- Carbon analyser TOC multi N/C 2100S, Analytik Jena
- Magnetic stirrers
- pH-meter 3310
- Heating chamber Memmert 4

Usage and, if applicable, calibration of all instruments following the corresponding SOP in the current edition. Standard laboratory glassware was also used.

7 PERFORMANCE OF THE STUDY

7.1 Preparations

The medium was prepared from the stock solutions. The stock solution of the positive control was prepared and its TOC was measured. The inoculum was taken from its source, washed, aerated and the dry matter was determined.

The test vessels were filled with medium and inoculum. Then all flasks were aerated for 72 hours with purified, CO_2-free, moistened air to purge the system of CO_2.

7.2 Experimental Parameters

Flask volume	1500 mL
Apparatus blanks	2, containing mineral medium only
Controls	2, containing mineral medium and inoculum
Positive control flasks	2, containing positive control, mineral medium and inoculum
Test flasks	2, containing test item, mineral medium and inoculum
Abiotic control	1, containing test item, mineral medium and $HgCl_2$
Toxicity control	1, containing test item, positive control, mineral medium and inoculum
Inoculum concentration:	25.0 mg/L
Temperature	19.0 – 20.5 °C
Duration	28 days

The test was performed with a nominal start concentration of 20 mg organic carbon/L.

The following amounts of test item and positive control were added to the flasks:

Table 7.2-a Amounts of test item and positive control in the flasks

Flask	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
Amount [redacted] in mg / L	--	--	137.5	137.7	139.1	138.3
Amount Aniline in mg / L	25.2	25.2	--	--	--	25.2
organic C (calculated) in mg / L	20.0	20.0	20.1	20.1	20.3	40.2

7.3 Apparatus

The test vessels were aerated with purified (by activated charcoal), CO_2-scrubbed, moistened air. The scrubbing of carbon dioxide was achieved by bubbling the purified air through a flask containing 1.5 m-NaOH. To control the absence of CO_2, the air was then led through a flask containing a solution of $Ba(OH)_2$ before reaching the test vessels.

Magnetic stirrers were used to prevent deposition of inoculum.

The emitted CO_2 was trapped in 0.25-m-NaOH. Two scrubbers containing 100 mL each were connected in series to the test vessels. The initial IC value of the 0.25 m-NaOH was separately determined in each flask.

7.4 Sampling

From each front scrubber flask, ten samples were taken in order to determine the emitted CO_2 (on days 0, 2, 4, 7, 9, 11, 15, 18, 23 and 29). The sample volume was 1 mL. The resulting change in the volume of the front flask was considered in the calculation of emitted CO_2 (see also chapter 8.3.1).

On day 28, 5 mL HCl 2-m. were added to each test flask in order to drive off dissolved CO_2. On day 29, samples from both scrubber flasks were taken.

7.5 CO_2 Determination

Analyses of the emitted CO_2 were made by IC measurement using the carbon analyser TOC multi N/C 2100S, Analytik Jena. Each sample was measured at least in duplicate. The carbon analyser was calibrated with freshly prepared reference solutions once a week. After every start, quality control samples were measured.

8 FINDINGS

8.1 Tables

8.1.1 IC-Values

In the following tables, the IC values (given in mg/L) which were measured in the samples of the front scrubber flasks are stated.

Table 8.1-a IC Values in mg/L Apparatus Blanks, Controls, front scrubber

Day	Apparatus blank 1	Apparatus blank 2	Control 1	Control 2
0	4.25	3.88	4.12	4.31
2	5.89	6.81	9.33	10.35
4	6.48	7.53	18.79	17.59
7	11.77	11.38	31.79	29.63
9	12.27	12.75	35.48	34.42
11	13.51	12.02	42.45	38.36
15	18.99	16.18	52.48	47.24
18	20.54	15.71	59.10	56.15
23	21.86	18.08	62.52	58.91
29	30.09	30.59	77.40	78.83

Table 8.1-b IC Values in mg/L Positive Control, Test Flasks, front scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	4.19	3.67	3.46	3.63	4.19	4.12
2	10.23	12.12	12.75	8.16	7.90	12.79
4	61.78	92.45	20.24	15.28	9.24	99.90
7	218.45	212.41	30.52	24.76	12.54	183.97
9	261.48	254.69	35.95	31.24	13.87	223.52
11	290.43	294.41	40.46	36.54	15.17	243.57
15	321.11	318.33	48.12	41.79	18.27	266.81
18	319.90	324.07	58.79	49.53	21.26	284.78
23	344.97	342.11	59.12	51.97	22.86	288.03
29	354.06	355.45	71.36	71.51	27.96	309.49

In the following tables, the IC values which were measured in the samples of the back scrubber flasks are stated.

Table 8.1-c IC Values in mg/L Controls, Apparatus Blanks, back scrubber

Day	Apparatus blank 1	Apparatus blank 2	Control 1	Control 2
0	3.57	4.38	4.23	4.46
29	8.10	5.57	8.11	6.57

Table 8.1-d IC Values in mg/L Positive Control, Test Flasks, back scrubber

Day	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	4.19	4.61	4.29	4.92	4.11	4.35
29	8.17	6.69	5.90	6.84	10.93	5.63

8.1.2 Net IC

For each flask, the IC value which was measured at the start of the test (d = 0) was subtracted from all following measurements. The net IC was calculated using this corrected measurement value and subtracting the mean IC value of the apparatus blanks of that sampling date.

The net IC values are presented in the following table.

Table 8.1-e Net IC-values in mg/L front scrubber flasks

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	4.1	4.3	4.2	3.7	3.5	3.6	4.2	4.1
2	7.0	8.1	7.9	9.8	10.5	5.9	5.6	10.5
4	15.9	14.7	58.8	89.5	17.3	12.3	6.3	97.0
7	24.3	22.1	210.9	204.9	23.0	17.3	5.0	176.5
9	27.0	26.0	253.0	246.2	27.5	22.8	5.4	215.1
11	33.8	29.7	281.7	285.7	31.8	27.8	6.5	234.9
15	39.0	33.7	307.6	304.8	34.6	28.3	4.8	253.3
18	45.0	42.1	305.8	310.0	44.7	35.5	7.2	270.7
23	46.6	43.0	329.1	326.2	43.2	36.1	7.0	272.1
29	51.1	52.6	327.8	329.2	45.1	45.2	1.7	283.2

Table 8.1-f Net IC-values in mg/L back scrubber flasks

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
0	4.2	4.5	4.2	4.6	4.3	4.9	4.1	4.4
29	5.3	3.7	5.3	3.8	3.0	4.0	8.1	2.8

Negative values occur, when the apparatus blank was higher than the respective treatment. As the measured values in these blanks as well as in the abiotic control are very low, measurement uncertainties lead to negative degradation values in the abiotic control.

8.1.3 pH

In the following table, the pH at the end of the test (before addition of HCl) is given:

Table 8.1-g pH Test flasks on day 28

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
28	7.5	7.5	7.4	7.4	7.3	7.3	6.4	7.4

8.2 Equations

8.2.1 Emitted Carbon in mg/L

Emitted Carbon in mg/L test solution at time t is calculated using the following equation:

$$emittC = \frac{(IC(t) - IC(0)) * VolNaOH(t)}{VolTestVessel}$$

with

emittC	emitted carbon in mg/L test solution
IC(t)	inorganic carbon in mg/L NaOH at time t
IC(0)	inorganic carbon in mg/L NaOH at the start of the test
VolNaOH (t)	remaining volume NaOH in L in the scrubber at time t (Volume at t = 0 (here: 0.1 L) - ∑ (all sample volumes up to time t))
VolTestVessel	test vessel volume in L (here: 1.5)

For day 29, the IC content of both scrubber flasks was taken into account.

Calculation of emitted carbon is necessary for the assessment of validity. The value obtained with this equation is multiplied with 3.667 (44/12) in order to obtain emitted CO_2.

8.2.2 Degradation in %

The percentage biodegradation in the test flasks was calculated from:

$$\% \text{ degradation} = \frac{\text{emitted C (Test) in mg/L - Mean emitted C (Controls) in mg/L}}{\text{added C in mg/L}} * 100$$

Degradation in positive control and toxicity flasks was calculated analogously.

Abiotic degradation was calculated from:

$$\% \text{ degradation} = \frac{\text{mg emitted C (abiotic)}}{\text{added C in mg}} * 100$$

8.3 Calculation Results

8.3.1 Emitted Carbon in mg/L

In the following table, the calculated emitted carbon (from IC values given in chapter 8.1.2 and equation stated in 8.2.1) "Equations "Emitted Carbon in mg/L" 8.2.1) is presented.

Table 8.3-a Emitted carbon in mg/L

Day	Control 1	Control 2	Positive Control 1	Positive Control 2	Test 1	Test 2	Abiotic Control	Toxicity Control
2	0.19	0.25	0.25	0.41	0.46	0.15	0.09	0.42
4	0.77	0.68	3.57	5.61	0.90	0.57	0.14	6.07
7	1.30	1.15	13.37	13.01	1.26	0.88	0.05	11.14
9	1.47	1.39	15.93	15.52	1.54	1.23	0.08	13.50
11	1.88	1.61	17.58	17.86	1.79	1.53	0.14	14.61
15	2.18	1.84	19.01	18.87	1.95	1.54	0.04	15.61
18	2.54	2.34	18.70	18.99	2.56	1.97	0.19	16.53
23	2.61	2.37	19.93	19.78	2.44	1.99	0.17	16.44
29	2.92	2.88	19.71	19.70	2.44	2.46	0.11	16.83

8.3.2 Degradation Values

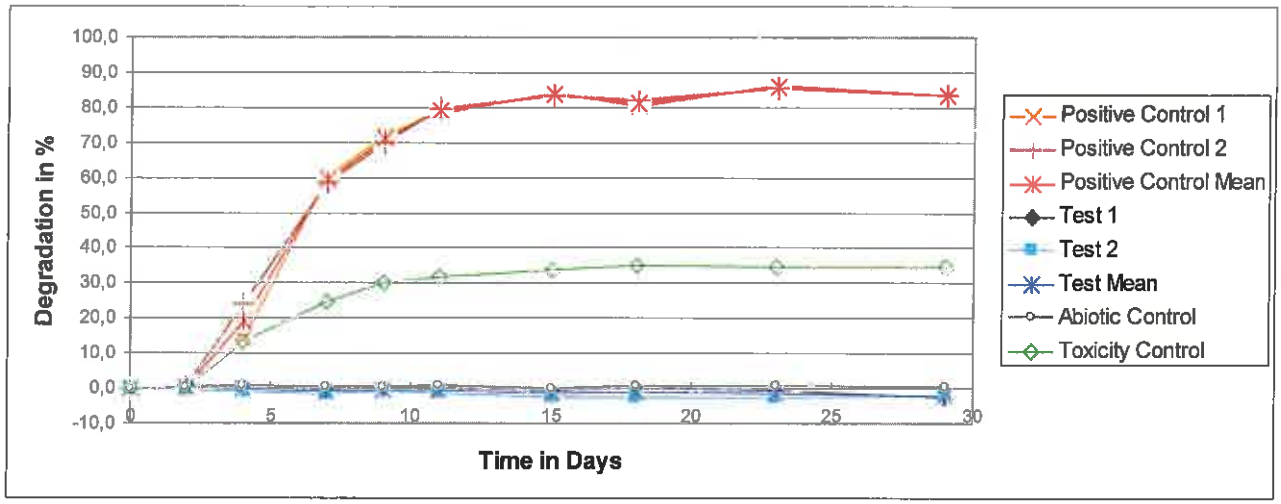
In the following table the percentage biodegradation is presented:

Table 8.3-b Degradation values in %

Day	Positive Control 1	Positive Control 2	Positive Control Mean	Test 1	Test 2	Test Mean	abiotic Control	Toxicity Control
2	0.1	0.9	0.5	1.2	-0.4	0.4	0.5	0.5
4	14.2	24.4	19.3	0.9	-0.8	0.1	0.7	13.3
7	60.7	58.9	59.8	0.2	-1.7	-0.8	0.3	24.7
9	72.4	70.4	71.4	0.6	-1.0	-0.2	0.4	30.0
11	79.1	80.5	79.8	0.3	-1.0	-0.4	0.7	32.0
15	84.9	84.2	84.6	-0.3	-2.3	-1.3	0.2	33.8
18	81.2	82.7	82.0	0.6	-2.3	-0.9	0.9	35.0
23	87.1	86.4	86.7	-0.3	-2.5	-1.4	0.8	34.7
29	84.0	83.9	83.9	-2.3	-2.2	-2.2	0.5	34.6

Since the values of day 29 are obtained from the addition of the IC values in scrubber flasks A and B, an increase (IC values in flasks B of the test higher than in those of the control) or a decrease (IC values in flasks B of the test lower than in those of the control) of degradation can be observed.

8.3.3 Degradation Graph



9 RESULTS AND VALIDITY

9.1 Results for the Test Item [redacted]

- The test item [redacted] is considered as "**not readily biodegradable**".
- No biodegradation was observed within 28 days.
- No 10-day-window was detected. The pass level of 60 % given in the OECD guideline was missed.
- The abiotic degradation was less than 1 %.

9.2 Validity

All validity parameters and values are presented in the following table:

Table 9.2-a Validity

Parameter	Criterion	Found	Assessment
IC content of test item solution in medium	≤ 5% of TC	< 1%	valid
CO_2 emitted by the controls	< 70 mg/L	10.6 mg/L	valid
Difference within replicates	≤ 20%	0.1 %	valid
Degradation of positive control > 60%	< 14 days	9 days	valid
Degradation in the toxicity flask on day 14	> 25%	32 %	valid

10 Discussion

All validity criteria were met.

Degradation behaviour of positive control and toxicity control was normal. Abiotic degradation was less than 1 %. Both replicates of the test item and the positive control showed very good correspondence.

If degradation in the toxicity flask is below 25% after 14 days, the test item can be considered as toxic towards the inoculum. As degradation in the toxicity flask was 32% after 14 days, the test item can be stated as "not toxic towards the inoculum in a concentration of 138.3 mg/l".

The test item [redacted] can be considered as "**not readily biodegradable**" because no degradation could be observed within 28 days

The result of the test can be considered valid.

11 Deviations

11.1 Deviations from the Study Plan

The following deviation from the study plan was documented:

- The temperature was a slightly lower than demanded in the study plan (19.0 – 20.5°C). As all validity criteria were met, this deviation can be stated as uncritical.

The deviation was signed and assessed by the study director on [redacted]

11.2 Deviations from the Guideline

See above.

12 Recording

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (shipment of documentation to sponsor). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1

13 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance
(gem. /according to § 19 Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

Prüfeinrichtung / Test facility

[redacted]

Prüfung nach Kategorien / Areas of Expertise
(gem. / according ChemVwV-GLP Nr. 6.3/OECD guidance)
1, 3, 4, 5, 6, 8

Datum der Inspektion / Date of Inspection
(Tag.Monat Jahr / day.month year)
27. und 28. November 2006

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.
Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

11.01.2007

Dr.-Ing. Karl-Heinz Rother - Präsident -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7
55116 Mainz
(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht

[redacted]

14 ANNEX 2: GLOSSARY

IC	inorganic carbon
OC	organic carbon
DOC	dissolved organic carbon
TOC	total organic carbon
TC	total carbon